Quantitative Proteomics in Neurodegenerative Disorders

Stephanie M. Cologna, Ph.D.
Department of Chemistry, University of Illinois at Chicago
Neurodegenerative Disorders

• Class of diseases with progressive neuron loss

• Degeneration of the central nervous system

• Neurodegenerative disorders include:
  • Alzheimer
  • Parkinson
  • Huntington
  • Lysosomal storage disorders

• Oftentimes, mis-folded proteins, fibrillation or deposits are observed
Cholesterol

• In mammals, synthesized enzymatically from acetyl-CoA and obtained from the diet peripherally

• Brain cholesterol is synthesized endogenously and tightly regulated

• Required for cell membrane structure and function

• Cholesterol has been implicated in neurodegenerative disorders
Niemann Pick Disease, Type C (NPC)

- A fatal, autosomal recessive neurodegenerative disease

- Accumulation of unesterified cholesterol and glycosphingolipids in the endosomal/lysosomal system

- Due to mutations in the *NPC1* or *NPC2* gene

  Mutations in the *NPC1* gene account for approximately 95% of diagnoses
NPC Neuropathology

• Neuronal apoptosis in the cerebral cortex and cerebellum

• Progressive neuronal loss, especially of cerebellar Purkinje neurons

• Neuron degeneration mechanism is unclear

• Some pathological overlap with Alzheimer Disease, such neurofibrillary tangles and tau hyperphosphorylation
Clinical Presentations of NPC

- Large variation in age of onset, and severity

- NPC has a wide spectrum of clinical presentations including **neurological** symptoms such as:
  - Ataxia
  - Progressive dementia
  - Seizures
  - Vertical gaze palsy

- Currently no FDA-approved therapy for NPC
  - Off-label miglustat, Gaucher Disease
  - Clinical Trial: 2-hydroxypropyl-β-cyclodextrin
Proteomic Studies in NPC1


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Cerebrospinal Fluid markers: Discovery-based quantitative proteomics

**Goal:**

Identify protein biomarkers in NPC1 CSF samples that provide insight to the neurodegenerative processes and can be used to follow therapeutic interventions

- Large sample cohort
- Several variables
- Small sample volumes
Beyond 8: iTRAQ Analysis of NPC1 CSF Samples

COHORT I

COHORT II

Albumin Depletion → Sample Cleanup and Quant → Trypsin Digestion → iTRAQ Labeling → Strong Cation Exchange → LC-MS/MS

I.S. #1

I.S. #2

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iTRAQ Proteomics Workflow

MS/MS Example Spectrum from A1AG1_HUMAN

Cohort 1  Cohort 2  Cohort 3

- Mascot
- Scaffold
- 25 Differential Proteins (≥ 2 fold p < 0.01)

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Optimization of Isobaric Tag Analysis

MSMS Spectra Using Standard DDA Method

MSMS Spectra Using Optimized DDA Method for Isobaric Tagged Samples (Chip Cube TMT)

MSMS Spectra Using Optimized DDA Method for Isobaric Tagged Samples (Jet Stream iTRAQ)

Optimized Collision Cell Voltages via increased slope

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Towards Label-Free Proteomics

AJS-ESI

HPLC-Chip/MS

NanoESI

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<th>NanoESI (2μg)</th>
<th>HPLC ChipCube (2μg)</th>
<th>AJS-ESI 2μg</th>
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Label-Free Quantitation in NPC1 Mouse Cerebellum

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Unfractionated

Technical Replicates Overlap

309 Proteins (FC ≥ 2)
Mouse Cerebellum Label Free Analysis of 20 Fractions

Sample preparation via FASP → High pH reverse phase fractionation → Database search in Spectrum Mill → Differential protein analysis in MPP

2784 proteins with ≥2 fold change

366
1120
655
643

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Summary and Outlook

- NPC1 is a fatal neurodegenerative disease without an approved therapy
- CSF-based proteomics has been carried out on individual samples
- Optimized method for reporter ion-based MS/MS data acquisition
- Explored Jet Stream platform for relative quantification and biomarker studies in NPC1
Acknowledgements

Cologna Laboratory – UIC

Fernando (Ralph) Tobias
Chathurika Rathnayake
Melissa Pergande
Thu (Mi) Nguyen

Agilent Technologies

Carol Haney-Ball, PhD
Vadi Bhat, PhD
Chris Klein, PhD
Ben Owen, PhD
Aaron Boice, PhD
Bob Walker
Mark Hoppe
Christine Miller
Maryalice Lundquist

NICHD, NIH

Forbes (Denny) Porter, MD, PhD
Alfred L. Yergey, PhD
Paul S. Blank, PhD

Porter Research Group

UIC Department of Chemistry

University of Illinois at Chicago

College of Liberal Art and Science

Agilent Technologies

NIH

Eunice Kennedy Shriver National Institute of Child Health and Human Development

NPD

Nina Dana Distal Arterial Lesions Foundation Inc.